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TECHNICAL MANUSCRIPT 88

# THERAPY IN EXPERIMENTAL COCCIDIOIDOMYCOSIS

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TECHNICAL MANUSCRIPT 88

THERAPY IN EXPERIMENTAL COCCIDIOIDOMYCOSIS

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### ABSTRACT

Campbell and Hill (1959) successfully used Fungizone, the presolubilized form of Amphotericin B intended for intravenous therapy, as an oral treatment for experimental coccidioidomycosis in mice. Their work was extended to further study the effects of dose level, length of therapy, and time of initiation of therapy on the course of the disease. A detailed histopathological study was made to compare changes in treated and untreated animals and to uncover possible renal damage resulting from the antibiotic.

An oral dose (in the drinking water) of 12 milligrams per kilogram per day, for 20 days, initiated at the time of IP challenge of mice with 1500 Coccidioides immitis arthrospores, resulted in almost no evidence of disease and the absence of positive cultures five months after challenge. Initiation of therapy as late as five or seven days postchallenge exerted a very beneficial effect on the course of the disease. However, per cent mortality, histological changes, and number of animals exhibiting positive cultures of the organism increased in direct proportion to the delay in treatment.

No histological evidence of renal damage was noted, even in animals receiving total oral doses of Fungizone as high as three grams (150 milligrams per day for 20 days).

## THERAPY IN EXPERIMENTAL COCCIDIOIDOMYCOSIS

Unsuccessful treatment of disseminated coccidioidomycosis is believed to result from inability of the various drugs to gain access to the organism after caseous, walled-off abscesses have formed in the tissues. Amphotericin B, the most promising antibiotic for treatment of this disease, is not only toxic by intravenous injection, but also very insoluble in the form prescribed orally. Campbell and Hill\* found that if the presolubilized product (Fungizone), intended for intravenous injection, were placed in drinking water, serum levels of 1.2 micrograms per milliliter could be demonstrated in mice\*\* after oral consumption of only 100 milligrams per kilogram. Furthermore, total dosages of as little as 70 milligrams per kilogram prolonged the survival of infected mice and produced negative cultures following intravenous challenge with Coccidioides immitis, Histoplasma capsulatum, or Cryptococcus neoformans.

An extension of this work was initiated to include histopathological studies of Amphotericin B-treated mice that had been infected with C. immitis. Two groups of mice were inoculated intraperitoneally with 150 or 1500 arthrospores of C. immitis, strain Silveira per animal. Each group was divided into three subgroups of 20 mice each, and subjected to a 10-day oral treatment with Fungizone at theoretical levels of 7, 14, or 28 milligrams per kilogram per day, initiated on the seventh day postchallenge. Treated, unchallenged controls for each dose level of antibiotic were maintained, along with untreated challenged controls. Animals were observed for a five-month period. Each animal that died from the infection and three animals sacrificed from each group at 30, 60, 90, 120, and 150 days were cultured and subjected to a complete necropsy. All surviving animals were sacrificed at five months and the same procedures were followed.

No deaths occurred among the treated animals until approximately two weeks after treatment was discontinued. At that time, 60 per cent of the untreated mice were dead. Histopathological studies of the untreated controls indicated severe involvement of the lungs, kidney, liver, spleen, and heart (Figure 1).

Fifty per cent of the treated animals sacrificed at 30 days (10/18) showed very mild to moderate histopathological changes, particularly in the lungs, although not nearly to the extent found in the untreated animals. Eight of this group were not infected. Approximately 70 per cent of the treated animals sacrificed at 60 days (13/18) showed histopathological changes, although the lesions were of a more chronic nature. Three of these animals were not infected, and two others were healed (Figure 2).

\* Campbell, C.C., and Hill, G.B. "Beneficial therapeutic effects of solubilized Amphotericin B following oral administration in experimental coccidioidomycosis, histoplasmosis, and cryptococcosis in mice," Trans. 4th Ann. VA-AF Coccidioidomycosis Study Group, 1959.

\*\* In conducting the research reported herein, the investigators adhered to "Principles of Laboratory Animal Care" as established by the National Society for Medical Research.



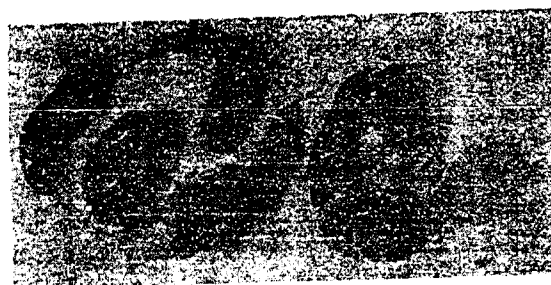
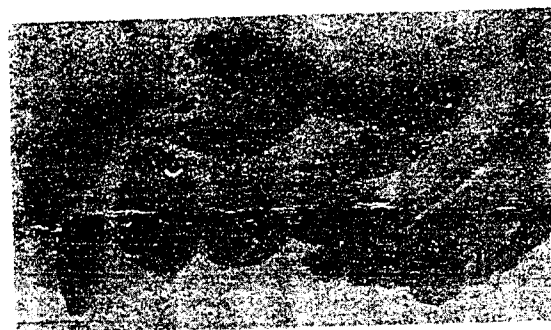


Figure 1. Top, Normal Mouse Tissue. Note clear lung. All others, challenged, untreated mice; note extensive destruction of lung tissue.

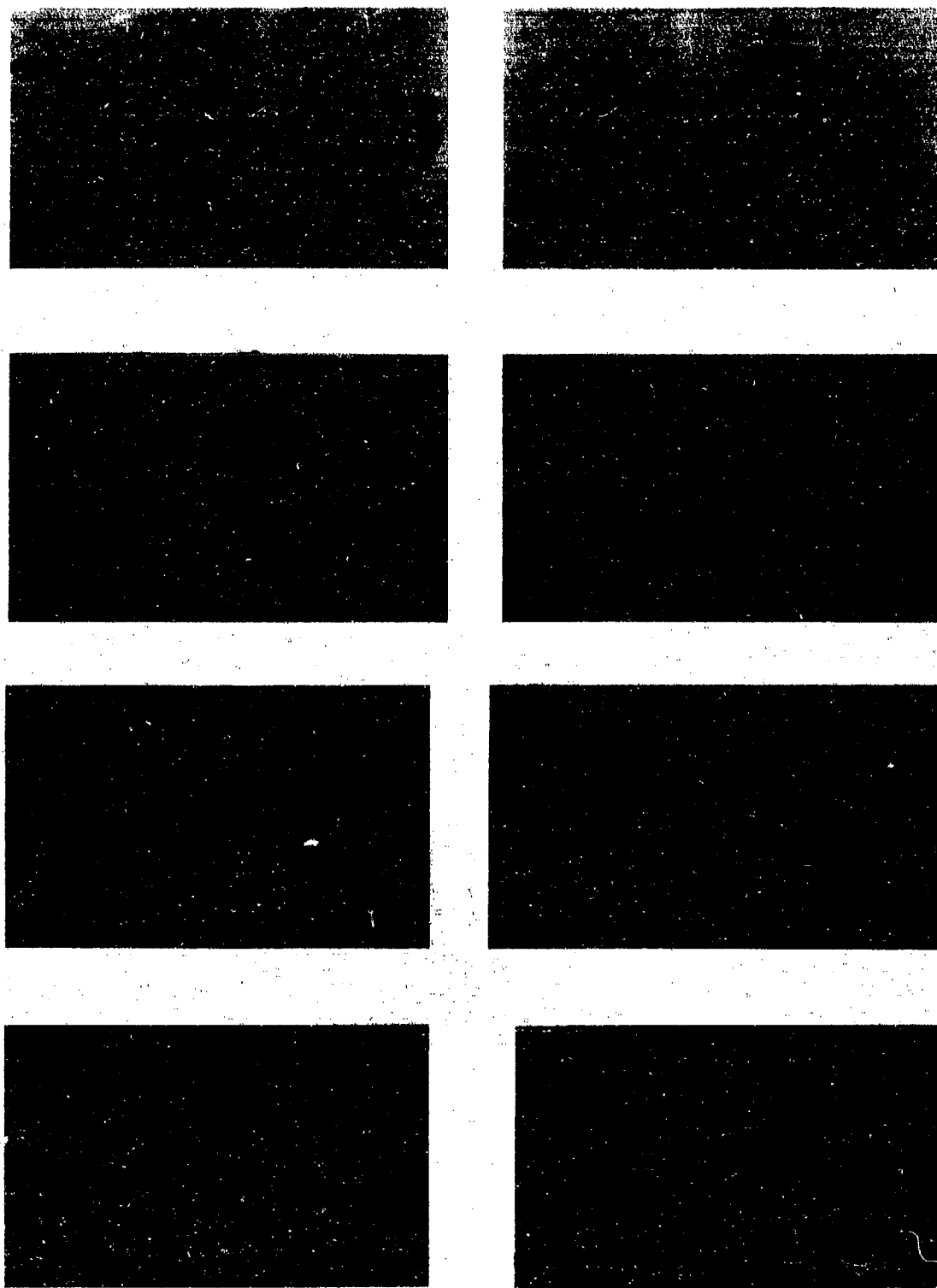


Figure 2. Top Left, Normal Control. Top Right, Challenged, Untreated Control. All Others, Challenged, Treated mice.

The following conclusions were drawn from a consideration of all data at the end of five months. No significant difference in survival was noted (a) among animals receiving the three treatment dose levels or (b) between treated animals receiving the low or high challenge dose. Seventy-four per cent of the treated animals were alive five months after challenge, as compared with only seven per cent of the untreated controls.

Similarly, no difference in extent of histopathological changes was noted among animals receiving the three treatment dose levels; however, the pathological involvement was greater in those receiving the higher challenge dose. As shown by serial sacrifice at monthly intervals, the tissue changes in all groups of treated animals increased with time, following cessation of treatment. The treated animals exhibited approximately 50 per cent less tissue involvement than the controls (Table I), although the infection in a significant number of them appeared walled off and well-controlled, and in a few instances complete healing of the infection was noted. Also, as shown in Table I, more than 80 per cent of the untreated animals exhibited lesions in three to five organs. This was in contrast to the treated mice in which approximately 70 per cent either had no visceral lesions or the infection appeared in only one or two organs. Cultural studies indicated that approximately 30 per cent of the survivors were still harboring the organism five months after challenge.

These data indicated the necessity of an extension of the 10-day treatment or initiation of treatment earlier than seven days postchallenge. Further experiments were initiated, using a dose of 12 milligrams per kilogram per day of Amphotericin B over a period of 20 days, initiated one day prior to challenge, five days postchallenge, or seven days postchallenge, with an intraperitoneal challenge dose of 1500 arthrospores.

This regimen of treatment reduced the number of positive cultures and the extent of histopathological changes, and increased the five-month survival of treated animals. As shown in Table II, the per cent mortality, the extent of pathological changes, and the number of positive cultures all increased in direct proportion to the delay in treatment; those in the group receiving immediate therapy were essentially uninfected. The difference between a 10- and a 20-day treatment is indicated by the values in parenthesis.

An illustration of the terms mild, moderate, and severe histopathological changes indicated in Table II are shown in Figure 3.

TABLE I. HISTOPATHOLOGICAL INVOLVEMENT

Organs Affected	Untreated, %	Treated, %	No. of Organs Affected	Untreated, %	Treated, %
Lung	95	69	0	5	14
Spleen	85	44	1	5	37
Liver	80	28	2	10	21
Kidney	38	11	3	35	11
Pancreas	10	0	4	35	7
Other (Heart, Testes, etc.)	9	1.5	5	13	5

TABLE II. THERAPY WITH AMPHOTERICIN B<sup>a</sup>/

Initiation of Treatment	Number of Deaths	Per Cent Mortality	Histopathological Changes	% Positive Cultures
-1	2/49	4	None	0
+5	4/49	8	Minimal	13
+7	8/48	17	Moderate	38
(+7) <sup>b</sup> /	(13/48)	(26)	(Moderate to Severe)	(50)
Untreated	22/22	100	Severe	100

a. Twelve milligrams per kilogram per day for 20 days.

b. Figures in parentheses: Mice receiving a 10-day treatment.

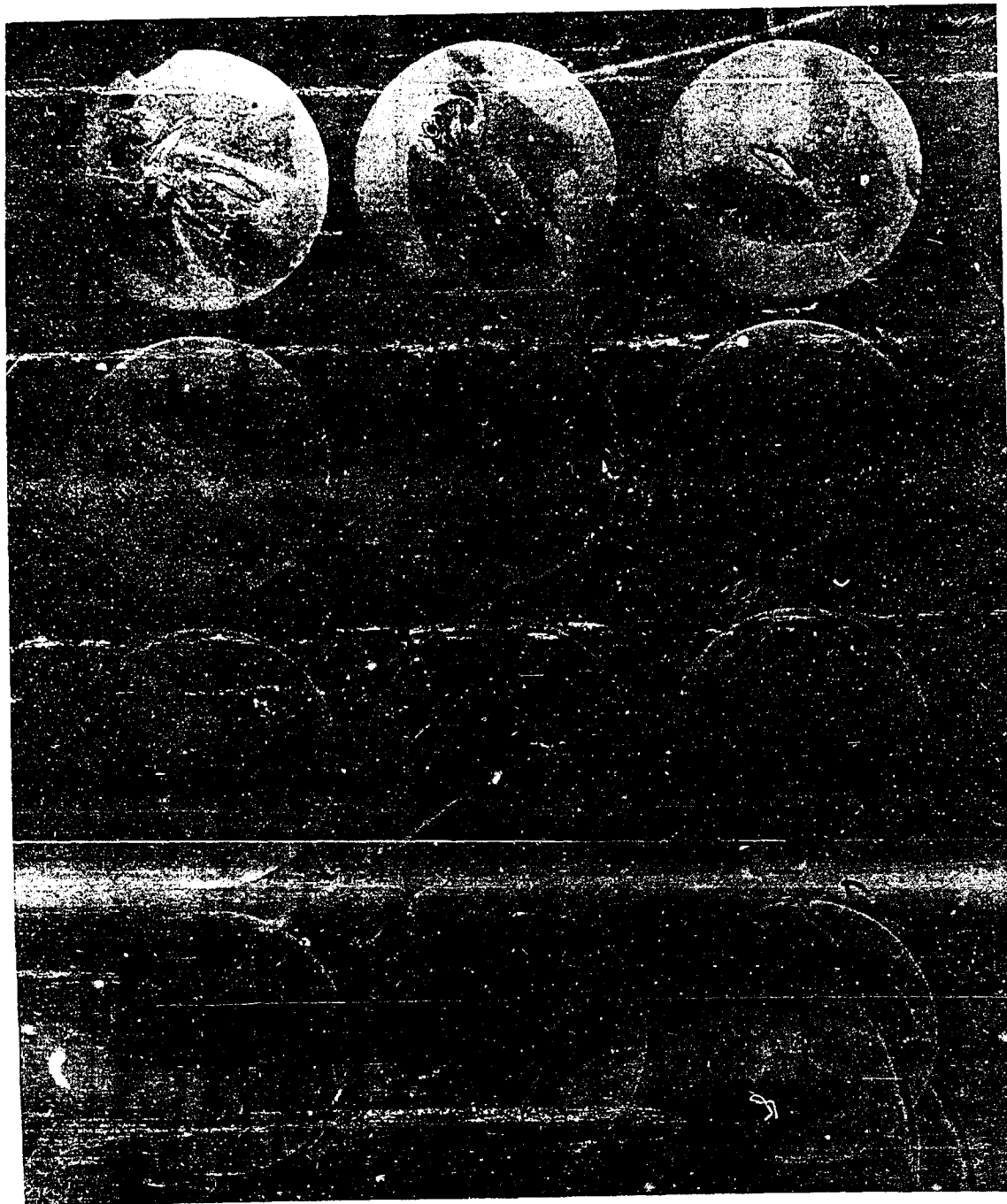


Figure 3. Illustrative Slide Showing Degrees of Histopathological Changes. Left to right, severe, moderate, mild, and negative.

In similar studies with mice, Amphotericin B was replaced in the drinking water by various compounds such as other antibiotics, sulfa drugs, iodides, etc. These substitutions did not result in significant modification of the disease. Further experiments were carried out, to study the combined effects of Amphotericin B therapy and immunization with a formalin-killed, arthrospore vaccine. The combination of these two regimens did not appear additive in extending survival; however, fewer positive cultures resulted and lung lesions appeared smaller and more self-contained in animals receiving both immunization and therapy.

In summary, the oral treatment of mice with Amphotericin B at a dose level of 12 milligrams per kilogram per day for 20 days, initiated at the time of exposure to C. immitis, resulted in almost no evidence of disease, and no recovery of the organism from the tissues five months after exposure. The initiation of therapy as late as five to seven days after exposure to the organism exerted a very significant beneficial effect on the course of the disease. Percent mortality, extent of histopathological changes, and number of positive cultures of mice increased in direct proportion to the delay in treatment.

Immunization in combination with Amphotericin therapy, although not extending survival beyond that of therapy alone, did result in fewer positive cultures and smaller, more focalized lung lesions.

It is interesting to note, in light of evidence presented at this meeting last year concerning renal damage from intravenous therapy, that not a single instance of renal damage was noted in mice receiving oral Amphotericin therapy. Admittedly, the total dose in mice per animal was small, 0.5 to 11 milligrams, but in similar studies with dogs, total oral doses as high as three grams also have failed to result in any clinical or histopathological evidence of nephrotoxicity.

It is felt that oral treatment of humans with the presolubilized form of Amphotericin B might be of value following unusual, known exposure to C. immitis, such as laboratory accidents with the organism. Although the extreme bitterness, and disagreeable side effects, of the solvent (deoxicholate) in Fungizone may preclude its oral use in humans, undoubtedly another solvent could be found for the antibiotic.